Result Page

The invention concerns Peptid preparations and procedures for their production.

With the search for effective contents materials from animal fabrics for application in the medicine, biotechnology and Kosmetik in the past decades a large number of substances and substance mixtures were extracted, characterized completely or partially and successfully used. On behalf only some few substances are mentioned, like Epidermal Growth Factor, Kollagen type I and type III and Hyaluronsäure (British Medical bulletin, volume. 45, No. 2, 1989, ?Growth Factors?, OD. M. D. Waterfield, Churchill Livingstone, Edinburgh; Collages in Health and Disease, eds. J. B. Knows and M. I. V. Jayson, Churchill Livingstone, Edinburgh, 1982; Methods in Enzymology, volume. 82, 1982, ?Structural and Contractile of protein, part A?, eds. L. W. Cunningham and D. W. Frederiksen, volume. 144, 1987, ?Structural and Contractile of protein, part D?, OD. L. W. Cunningham, Academic press, Inc., Orlando).

Many of the active substances found so far are very complex molecules, like proteins, Polypeptide or Mucopolysaccharide, which are synthesized from small components by the fabric cells. The goal of past efforts was essentially the extraction, with possibly following chemico-physical modification of these complex molecules, in order to then use it meaningfully.

By enzymatic or chemico-physical dismantling these macromolecules however usually lose their effect or experience effect losses or effect shifts. If for example Kollagen is diminished to gel, is reduced for the Kosmetik important water binding abilities [Parfümerie and Kosmetik 65 (7), 1984, 391 to 401, Alexander mountain: ?Use of proteins in the Kosmetik?; RAK - Smelling materials, flavours, Kosmetica (7), 1977, R. Riemschneider, W. H. Chik: ?Over the water binding ability of soluble Kollagene?]. With the further dismantling from gel to gel hydrolysate Peptide with a molecular weight are set free from 500 to 30,000 Dalton, which possess a better absorptive power for hair in the comparison to the gel and therefore within the range of the hair care attained great importance.

Article available invention is discovery that the won the Partialhydrolysate under defined conditions, which isolated from it Peptide and the mixtures of amino acids and Peptiden, whose amino acid sequences agree with the sections of the protein amino acid sequence, new effect characteristics possess, which go clearly beyond the effect of the intact proteins.

As example to Kollagen type I one refers briefly.

▲ top

Kollagene, from which at least 13 different types were isolated and characterized, predominantly serve as structure proteins in the connective tissue of multi-cellular organisms. Specific cells, like for example fibroblasts in the dermis, synthesize the needle-shaped protein molecules, which twisted with one another from three, cable-similar Polypeptiden are compound. With Kollagen two of the three Polypeptide identical amino acid sequences possess and become type I as alpha-1 (I) - designates Polypeptide (Fig. 4), while the third Polypeptid, alpha 2 (I) has, another amino acid sequence (Fig. 5). Each Polypeptid contains about 1000 amino acids. The entire protein has a length of approx. 0,3 mu m and a diameter of approx. 0,0015 mu M.

Because of the film-forming characteristics and the large water absorption ability Kollagen is used in important quantities in the Kosmetik. The cosmetic effect of Kollagen was so far essentially to these characteristics limited, due both by the chemical structure of the Kollagens and to the molecule size, which prevents a penetration into the skin.

If against it a Partialhydrolysat is manufactured by Kollagen under exactly defined conditions (see production preparation A) or a mixture from amino acids and Peptiden is prepared (production preparation B), whose amino acid synthesis corresponds to the kollagen amino acid analysis approximately and whose Peptide have amino acid sequences, which agree accurate with certain Kollagen Aminosäuresequenzen (Fig. 4 and 5), then exhibits these preparations new positive effects and characteristics.

These effects are partially in table 1 summarized. The effective preparations are called therein preparation B and GHL Peptid (GHL=Glycyl Histidyl-Lysin=Tripeptidfraktion A), and they are compared with the characteristics of the native Kollagens.

Table 1

Effect comparison between native Kollagen, preparation B and the GHL Peptid
EMI3.1

This Partialhydrolysate, isolated Peptide and preparations from amino acids and Peptiden is of special importance

1. for the biotechnology, when additive for cell culture nutritive solutions for serum-poor or defined, serum-free cell culture growth media (application example 1),
2. for the medicine, as wundheilungsförderndes means (application example 2), to the Immunstimulation and active substance for the increase of the body-own Erythropoetinbildung, and
3. for the Kosmetik, for the care of the skin, as anti- Aging factor and radical inhibitor complex (application examples 3 to 5).

Fundamental methods of the production

1. Synthetic production

The components of the prescription (tables 2 to 14: Prescription parliamentary groups) are in an appropriate way before-solved and mixed. Individual components of the prescription, like for example the Tripeptidfraktion A and Tripeptidfraktion B, are cleaned, isolated, concentrated after the Partialhydrolyse (fundamental methods of the production 3 to 5) with the help of chromatographischer methods and admitted afterwards to the preparation.

2. Halfsynthetic production

Components of the prescription are mixed with towards-technologically won substances and/or with Partialhydrolysaten (see fundamental methods of the production 3 to 5).

3. Partialhydrolysat with diluted hydrochloric acid

Animal skin or animal lung, Kollagen, gel or Elastin are hydrolyzed, as in requirement described 2, filtered, neutralized afterwards and entsalzt with the help of for example the reverse osmosis. If necessary a treatment with 1n-NaOH, one hour at room temperature, with following neutralization and renewed demineralization takes place.

4. Enzymatic Partialhydrolysat with Kollagenase from Clostridium histolyticum

This enzyme splits the repeating Gly X Y Gly x Y-Gly-X-Y-Gly-Aminosäuresequenz of the Kollagens between Y and glycine (X, Y stand for different amino acids in the Kollagen Aminosäuresequenz). Animal skin or animal lung and/or. Kollagen type I is treated in trichloroethylene HCl buffer, pH 7.6, in presence of CaCl2 90 minutes with 37 DEG C with Kollagenase and the solution is dialysiert, conserved and sterile-filtered afterwards 24 hours with +4 DEG C.

5. Partialhydrolysat of Hautkeratin with Pepsin

Animal epidermis is treated in the Citrat HCl buffer, pH 1.5, 24 hours at room temperature with

Pepsin (relationship Pepsin to substrate 1: 10) entsalzt, dialysiert, 24 hours at pH 10.5 at room temperature stored, 1 hour with 1n-NaOH at room temperature treated, neutralized, and sterile-filters.

6. Genetically won protein sequences

Suitable micro organisms are treated in such a way according to well-known techniques that them changed kollagen, Elastin and Hautkeratinmoleküle to synthesize, which contain a larger number of effective Peptidsequenzen. These changed proteins increase the yield with the production of effective Peptidsequenzen by partial hydrolysis.

7. Natural preparation

The natural preparation corresponds to the descriptions in accordance with requirement 2 and the fundamental methods of the production 3 to 5.

Table 2

Examples 1 to 6 (in each case g/L preparation)
EMI5.1

In the tables the 2 to 5 amino acids specified predominantly stands for amino acids of vegetable origin.

Table 3

Examples 7 to 12 (in each case g/L preparation)
EMI6.1

Table 4

Examples 13 to 18 (in each case g/L preparation)
EMI7.1

Table 5

Examples 19 to 24 (in each case g/L preparation)
EMI8.1

Table 6

Examples 25 to 30 (in each case mg/L preparation)
EMI9.1

The letters X, Y stand for amino acids of arbitrary kind and quantity from in each case 0 to maximally 50.

The Peptide in the tables 6 to 8 can be used as natural isolated Peptide and/or as synthetically hergetellte Peptide.

Table 7

Examples 31 to 36 (in each case mg/L preparation)
EMI10.1

Table 8

Examples 37 to 42 (in each case mg/L preparation)
EMI11.1

Table 9

Examples 43 to 48 (in each case mg/L preparation)
EMI11.2

Table 10

Examples 49 to 54 (in each case mg/L preparation

EMI11.3

Table 11

Examples 55 to 60 (in each case g/L preparation)
EMI12.1

Table 12

Examples 61 to 66 (in each case g/L preparation)
EMI12.2

Table 13

Examples 66 to 72 (in each case g/L preparation)
EMI13.1

Table 14

Examples 73 to 78 (in each case mu g, mg or g/L preparation)
EMI13.2

Production of preparation A

1 kg denatured Kollagen (gel) is suspended in 9 kg 1n-HCl-Lösung and fishing rod east. the solution is warmed up in a closely locked container under agitating fast to 100 DEG C, which neutralizes temperature 3 hours, afterwards fast constantly held ambient temperature cooled down and with 6n-NaOH-Lösung. The preparation becomes with the help of the gel chromatography and/or. other suitable methods entsalzt and/or with dest. Water filled up to a volume of 20 l, with 0,2% preservative (for example Phenonip or Hydroxybenzoesäureester) conserves and sterile-filters. Fig. 1 gives an overview of the amino acids and Peptide in the preparation to A. Fig. 1 is the result of a two-dimensional Dünnschichtchromatographie. As comparison Fig serves. 2 with a standard amino acid mixture, which was chromatographisch separated under the same conditions. Fig. 1A against it shows the amino acids and Peptide after insufficient partial hydrolysis.

The biological effectiveness of the preparation A was examined by determination of the metabolic activation at rat liver Mitochondrien. The preparation A caused a 60%ige metabolic increase in the comparison as a check.

For the stabilization of the Peptide the preparation A contained the prescription parliamentary group CAH-1 (table 11, example 55).

Production of preparation B

The preparation B contains the prescription parliamentary groups CAS-1, CTS-2, CAH-2, CMS-2 and CP-1.

0.6 kg distilled water submit and the easy-soluble amino acids first loosen. The heavysoluble amino acids (asparagine acid, glow amine acid, Leucin, Phenylalanin, Tyrosin, Valin) are before-solved in 2n NaOH solution. After addition of Citronensäure the pH value of the solution with 10n-NaOH is held within the range of 5 to 9 and adjusted after complete release of the Citronensäure to 6,5. Afterwards addition takes place from Mannit, glucose, Ascorbat, Natriumlactat, ethanol, Glycerin, Sojapeptiden, Dipeptiden, Tripeptiden, Tripeptidfraktion, trace elements and Hydroxybenzoesäuremethylester sodium salt. The pH value is stopped with 6n-HCl to 6,5. The beginning is dest with. Water to 1 l total beginning quantity filled up and under sterile conditions sterile-filters.

Isolation of the Tripeptidfraktion A and the Tripeptidfraktion B

The preparation A described above becomes preferably with highly soluble Säulenchromatographie, for example with silicagel 60 as sorbent and with Eluationslösungen, preferably Butanol/acetic acid/water 4: 1 : 1, and Propanol/ammonia (25%) 7: 3, in at least two Eluationsstufen isolated, in order to isolate a Tripeptid, which can be characterized analytically as follows:

After sour hydrolysis, 24 hours in 6n-HCl with 105 DEG C, are set free three amino acids, which have the following hRf values after linear Dünnschichtchromatographie: HRf values 34, 42, 11 for the amino acids 1 to 3 in the flow material 96% Ethanol/34% ammonia in the relationship 7: 3 and the hRf values 32, 20 and 2 for the amino acids 1 to 3 in the flow material 1 - Propanol/water in the relationship 7: 3. After the sequence analysis the numbering of the amino acids 1 to 3 corresponds to their positions in the Tripeptid, beginning with the aminoterminalen amino acid. Thus the Tripeptidfraktion A is identified as the Tripeptid Gly His Lys.

The appropriate steps are kept, in order to identify the Peptidfraktion B with the Tripeptid Gly Asp Ser. In place of the isolated Tripeptide also the appropriate Tripeptide cleaned snythetisierten in usual procedures and can be used.

Production of Peptid trace element complexes

A further process step with the production of the Peptidfraktion exists in the integration either the isolated or the synthesized Tripeptids with copper; 0,01 mol/L Tripeptid with 0,01 mol/L copper (II) - acetate mono hydrate mixed and with 0,1n-NaOH-Lösung neutralizes. The Tripeptidfraktion is coolly stored in small portions.

Effect proofs for the preparation A, preparation B and the Tripeptide

The biological effectiveness of the Tripeptids became and. A. at human skin fibroblasts in the cell culture attempt determines (Fig. 3). After addition from 10-8 to 10-11 mol/l Tripeptid to the cell culture growth medium the Kollagenproduktion was increased human skin fibroblasts by 50 to 250% in the comparison as a check.

The preparation B showed a metabolic increase in another biochemical investigation with liver Mitochondrien around 80% in the comparison as a check.

An easily varied preparation B, which contains the prescription parliamentary groups CAS-3, CP-5, CTS-3, CAH-3 and CMS-2, in addition causes a metabolic increase of 80% with liver Mitochondrien and possesses the ability to inactivate hydroxyl radicals up to 40% (Fig. 6).

In this specific quantitative radical inhibitor test by effect of the enzyme Xanthinoxidase are set free on the substrate Xanthin in a nuclear chain reaction highly reactive hydroxyl radicals. The viscose rayon Hyaluronsäure in the aqueous investigation solution is decomposed by the hydroxyl radicals within 40 minutes, measurably by the fast, strong viscosity waste. The measured value of the viscosity acceptance hangs off of the total quantity of the hydroxyl radicals and the quantity and quality possibly present hydroxyl radical inhibitor, which restrains the viscosity waste clearly.

Production of preparation C

The preparation C contains the prescription parliamentary groups AS-1, BP-2, BAH-1, BMS-1 and BTS-1.

0.6 kg distilled water are submitted and the easy-soluble amino acids are stirred. The heavysoluble amino acids are before-solved in 2n-NaOH. Guanine becomes in 3n-HCl under warming up to approx. 60 DEG C solved. The before-solved amino acids are stirred into the beginning. After addition of Citronensäure the pH value of the solution with 10n NaOH is adjusted, so that the pH value of 5 is not fallen below and 9 is not exceeded. After complete release of the Citronensäure the guanine HCl solution is stirred and the pH value is stopped to 6,5. Afterwards addition takes place from Mannit, Sorbit, Natriumlactatlösung, Glycerin, ethanol, Natriumascorbat, Sacchariden, Peptiden, nucleotides, Tripeptidfraktion, Hautpartialhydrolysat (related to the dry weight), trace elements and Hydroxybenzoesäuremethylester Natriumsalz.Der pH value with 6n-HCl to 6,5 is adjusted, the beginning with dest. Water to 1 L total volume filled up and under sterile conditions sterile-filters.

The Tripeptidfraktion corresponds to the above description (see production of the Tripeptidfraktionen A and B).

Production of the Hautpartialhydrolysats

Washed one and of subcutaneous fatty tissue released calf skin is cut up. 3 kg cut up fabric (damp

weight) in 7 kg 1,5n HCl is suspended, warmed up fishing rod east and in a closely locked container under agitating fast to 100 DEG C. After 3 hours with 100 DEG C fast ambient temperature is cooled down and neutralized with 10n-NaOH-Lösung. After multi-level filtration over depth layer filters the clarified excerpt with 0,2% Hydroxybenzoesäuremethylester sodium salt is conserved, which sterile-filters pH value of the solution adjusted to 6,5 and the solution under sterile conditions. According to determination of the dry weight an appropriate volume of the Hautpartialhydrolysats - related to the dry weight - is brought into the preparation.

The biological effectiveness of the halfsynthetic Bindegewebsextraktes was examined at rat liver Mitochondrien. An increase of the metabolic activity was measured around 80%.

Application examples 1 and 2

Biotechnology and medicine

Application example 1

Biotechnology

The stimulation of cell growth or the synthesis from Stoffwechselprodukten becomes serum-poor or - free cell culture media approx. 1 to 5% of the preparations described in this invention added. The cell culture nutritive solution for skin fibroblasts has the following composition: 94% Dulbecco's minimum Essential medium, inclusive. 2 mmol/l Glutamin,
5% fötales calf serum.

Application example 2

Medicine

The promotion of the Wundheilung become approx. 2 to 5% of the described preparations or at least 50 to 200 mg Gly His Lys/kg into medical ointments, creams, lotions, Tinkturen, Wundheilungssprays trained or Wundabdeckungen thereby impregnates.

Application examples 3 to 5

Kosmetik

At least three effect characteristics of the preparations described in this invention point to a successful application with the care of the skin: The increase of the metabolic activity, the radical inhibitor effect and the stimulation of the Kollagensynthese of fibroblasts.

The cosmetic preparations for the care of the skin: Humidity and day creams for dry skin, night creams, sun protection preparations, after Sun lotions, anti-fold creams, skin protection creams and after Shave lotions should contain a minimum concentration from 2 to 5% of the preparations described in this invention.

Application example 3

Humidity cream

<tb>< TABLE> Columns=2>
<tb> A.
<tb> Lanette 0< September> 3.0%
<tb> Isopropylmyristat< September> 4.0%
<tb> Abil AV 200< September> 1.0%
<tb> Arlacel 165< September> 6.5%
<tb> emulsifying agent G-1790< September> 3.8%
<tb> Propyl-4-hydroxybenzoat< September> 0.05%
<tb> Oxynex 2004< September> 0.02%
<tb> B. Allantoin< September> 0.3%
<tb> Karion F liquid< September> 6.0%
<tb> Methyl-4-hydroxybenzoat< September> 0.2%
<tb> water that.< September> 65.43%

<tb> C. Kollagen< September> 5.0%
<tb> preparation B< September> 3.0%
<tb> D. Perfume oil< September> 0.3%
<tb>< /TABLE>

Production

Phase A with 80 DEG C melt and phase B to 80 DEG C warm up. B under agitating A add. Stir the phases C and D with 30 DEG C.

Application example 4

Day cream for dry skin

<tb>< TABLE> Columns=2>
<tb> A.
<tb> Sonnenblumenöl< September> 5.0%
<tb> peanut oil< September> 5.0%
<tb> wheat germ oil< September> 5.0%
<tb> Sheabutter< September> 5.0%
<tb> Phenonip< September> 0.3%
<tb> B. Water, that.< September> 66.85%
<tb> D-Panthenol 50%ig< September> 1.0%
<tb> aloe vera< September> 2.0%
<tb> Phenonip< September> 0.3%
<tb> C. Preparation C< September> 3.0%
<tb> D. Perfume oil< September> 0.3%
<tb>< /TABLE>

Production

Phase A with 75 DEG C melt and phase B to 75.C warm up. B into A stir. C with 45 DEG C and D with 35 DEG C stir.

Application example 5

Sun protective creme

<tb>< TABLE> Columns=2>
<tb> A.
<tb> Cremophor WHERE 7< September> 2.0%
<tb> Elfacos sp 9< September> 2.0%
<tb> ISO Adipat< September> 12.0%
<tb> Permulgin 3220< September> 2.0%
<tb> vaseline knows< September> 5.0%
<tb> magnesium stearate< September> 0.5%
<tb> Aluminiumstearat< September> 0.5%
<tb> Isopropylmyristat< September> 10.0%
<tb> Uvinult 150< September> 3.0%
<tb> B. 1,2-Propylenglykol< September> 5.0%
<tb> Magnesiumsulfat-7-Hydrat< September> 0.7%
<tb> Phenonip< September> 0.25%
<tb> water< September> 45.75%
<tb> C. Preparation B< September> 3.0%
<tb> D. Perfume oil< September> 0.3%
<tb>< /TABLE>

Production

Phase A and phase B separately to 75 DEG C warm up and B into phase A slowly stir, homogenize and cold-agitate. C with 45 DEG C and D with 35 DEG C stir.